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Quercetin and Its Metabolites Inhibit Recombinant Human Angiotensin-Converting Enzyme 2 (ACE2) Activity

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ABSTRACT: Angiotensin-converting enzyme 2 (ACE2) is a host receptor for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Inhibiting the interaction between the envelope spike glycoproteins (S-proteins) of SARS-CoV-2 and ACE2 is a potential antiviral therapeutic approach, but little is known about how dietary compounds interact with ACE2. The objective of this study was to determine if flavonoids and other polyphenols with B-ring 3′,4′-hydroxylation inhibit recombinant human (rh)ACE2 activity. rhACE2 activity was assessed with the fluorogenic substrate Mca-APK(Dnp). Polyphenols reduced rhACE2 activity by 15−66% at 10 μM. Rutin, quercetin-3-*O*-glucoside, tamarixetin, and 3,4-dihydroxyphenylacetic acid inhibited rhACE2 activity by 42−48%. Quercetin was the most potent rhACE2 inhibitor among the polyphenols tested, with an IC_{50} of 4.48 μM. Thus, quercetin, its metabolites, and polyphenols with 3′,4′-hydroxylation inhibited rhACE2 activity at physiologically relevant concentrations *in vitro*.

KEYWORDS: *quercetin, angiotensin-converting enzyme 2, enzyme kinetics, polyphenols*

INTRODUCTION

Human angiotensin-converting enzyme 2 (ACE2) is an 805 amino-acid transmembrane protein that contains an extracellular domain with a typical HEMGH metalloproteinase zinc-binding site.[1,2] ACE2 is localized at the lung alveolar epithelial cells, arterial and venous endothelial cells, renal tubular epithelium, and the epithelia of the small intestine.[3−5] This protein acts as monocarboxypeptidase that exclusively cleaves a single C-terminal residue from angiotensin II (Ang II), generating angiotensin-(1-7).[6] It counterbalances the accumulation of Ang II formed by the action of the angiotensin-converting enzyme (ACE). The anti-inflammatory properties of ACE2 are mediated by the activation of the Mas receptor through the ACE2-Ang-(1-7)-Mas axis[7] in the renin−angiotensin−aldosterone system (RAAS).[7−9] More recently, ACE2 was identified as a receptor for S-proteins of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) to infiltrate host cells.[10−12] Inhibiting the interaction between S protein and the host ACE2 is predicted to inhibit SARS-CoV-2 infection.[13−15]

A preliminary computational molecular docking study identified quercetin, luteolin, and eriodictyol as potential inhibitors of the interaction between S protein and ACE2.[16] It is predicted that polyphenols inhibit the entry of SARS-CoV-2 into cells by restraining the viral S protein−human ACE2 interface; however, detailed studies that describe the interaction of flavonoids with ACE2 are scarce. It is evident from earlier studies that the kaempferol and polyphenol-containing vegetable extracts inhibit rhACE2 activity.[17] Quercetin, luteolin, and eriodictyol are structurally similar flavonoids having 3′,4′-hydroxylated B-rings. Therefore, we hypothesized that flavonoids and phenolic acids with B-ring 3′,4′-hydroxylation would inhibit ACE2 activity (Figure 1). Since polyphenols are extensively metabolized upon consumption, we further evaluated the potential for quercetin metabolites to also inhibit rhACE2. In this study, we describe the validation of an rhACE2 assay and the extent these polyphenols act as rhACE2 inhibitors.

MATERIALS AND METHODS (INCLUDING SAFETY INFORMATION)

Chemicals and Reagents. Purified polyphenols were obtained for inhibition assays. Quercetin was obtained from Tocris (Minneapolis, MN). Rutin, luteolin, and 3,4-dihydroxyphenylacetic acid were obtained from Sigma-Aldrich (St. Louis, MO). Quercetin-3-*O*-glucoside, quercetin-3-*O*-galactoside, quercetin-3-*O*-glucuronide, tamarixetin, isorhamnetin, (±)-eriodictyol, (−)-epicatechin, and nicotianamine were obtained from Cayman Chemical (Ann Arbor, MI). The ACE2 substrate Mca-APK(Dnp), ACE2-specific inhibitor DX600, and fluorescence standard Mca (7-methoxycoumarin-4-acetic acid) were obtained from AnaSpec (Fremont, CA). rhACE2 was obtained from R&D Systems (Minneapolis, MN). All other chemicals were obtained from Sigma-Aldrich unless otherwise indicated.

Determination of rhACE2 Enzyme Activity. The catalytic activity of rhACE2 was monitored using the substrate Mca-APK(Dnp), in which Mca fluorescence is quenched by Dnp until cleavage at Pro-Lys[18] separates them. Fluorescence intensity was measured in black 96-well, optical polymer base plates (Thermo Scientific Nunc, Rochester, NY) at 320 nm excitation and 405 nm emission, with 180 rpm continuous shaking using a fluorescence plate reader (Varioskan Flash, Thermo Scientific, Vantaa, Finland). The

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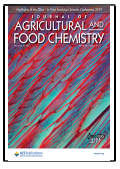

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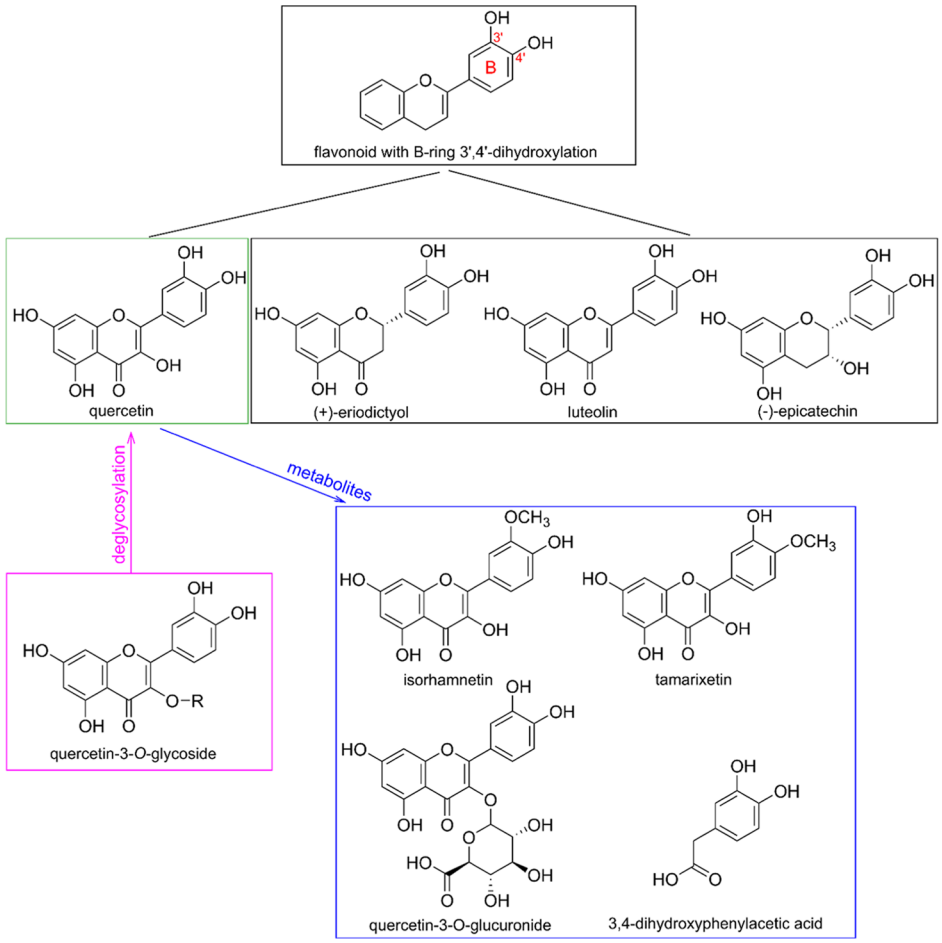

Figure 1. Quercetin and its related glycosides and metabolites, as well as flavonoids with B-ring 3′,4′-dihydroxylation were screened in this study. Quercetin primarily exists as glycosides in nature (*R* = glucose, galactose, or rutinose). These quercetin-3-*O*-glycosides are converted to quercetin by the host's gastrointestinal tract and further metabolized by the gut microbiota and host.

assay buffer used in the rhACE2 activity assay was made in-house, having 0.05 M 2-morpholinoethane-sulfonic acid (MES), 0.3 M NaCl, and 10 μM $ZnCl_2$, pH 6.8. It was stored at 4 °C when not in use. Polyphenol standards (quercetin, quercetin-3-*O*-glucoside, quercetin-3-*O*-galactoside, rutin, tamarixetin, isorhamnetin, (±)-eriodictyol, (−)-epicatechin, luteolin, and 3,4-dihydroxyphenylacetic acid) and nicotianamine were first dissolved in methanol with a concentration of 2 mM as stock solutions. A methanol vehicle affected assay kinetics, so to conduct experiments in the absence of methanol, aliquots of these stock solutions were evaporated at room temperature and then reconstituted in the assay buffer. The rhACE2 enzyme, the Mca-APK(Dnp) substrate, and DX600 stock solutions were further diluted in the assay buffer to working solutions. For accuracy, all of the reagents were prewarmed to assay temperatures for 10 min before addition to the microplate.

To assess rhACE2 activity and optimize assay conditions, rhACE2 was serially diluted in assay buffer with a starting concentration of 250 ng/mL. In a 100 μL total reaction volume, 40 μL of the enzyme solution was mixed with 60 μL of the Mca-APK(Dnp) substrate (11.25 μM final concentration). The mixture was incubated at 37 °C for 60 min. Background controls consisted of 40 μL of assay buffer with 60 μL of the substrate.

For inhibition assays, 20 μL of the inhibitor was coincubated with 40 μL of rhACE2 (50 ng/mL final concentration), and then 40 μL of the substrate (final concentration 11.25 μM) was added for 10 min, and the fluorescence was measured at 2 min intervals at 37 °C. For quercetin, activity was fitted as a variable slope [inhibitor]-normalized response model in GraphPad Prism 7.0 to determine the IC_{50} value. The mixture of 50 ng/mL rhACE2 and 11.25 μM substrate without quercetin exhibited the maximum rhACE2 activity. Inhibitors were screened by coincubating 50 ng/mL rhACE2 enzyme with 11.25 μM substrate with 10 μM of compounds or 1 μM of DX600 at 37 °C for 10 min, and the fluorescence was determined at 2 min intervals. Inhibitors were included in background control samples. The inhibition (%) was calculated by comparing the fluorescence with the maximum fluorescence as

$$\%\text{ACE2 activity} = [(A - B)/(C - D)] \times 100$$

where *A* is the fluorescence in the presence of the inhibitor, *B* is the background control (inhibitor + substrate) fluorescence, *C* is the fluorescence of the uninhibited rhACE2 control, and *D* is the background substrate control fluorescence.

Determination of Enzyme Kinetics. K_m, V_{max}, and K_{cat} were determined at 50 ng/mL rhACE2 with 0.7−90 μM substrate in the presence or absence of quercetin (10 μM), rutin (10 μM), or DX600 (1 μM) for 10 min at 37 °C. The initial velocity was determined by assessing the velocity over (0−10 min), using linear regression and a Mca standard calibration curve. K_m, V_{max}, and K_{cat} were estimated by fitting the velocity data to the Michaelis−Menten model in GraphPad Prism 7.0.

Mca Fluorescence and rhACE2 Enzyme Activity Standardization. Mca fluorescence reference standard curves were generated by coincubating 40 μL of Mca (0.0375−5 μM, final concentration) and 60 μL of the substrate at matching temperature and times of the assay conditions. Assay buffer (40 μL) with the substrate (60 μL) was used as a background control. The fluorescence was recorded at an excitation of 320 nm and emission of 405 nm. A plot of relative fluorescence units (RFU) versus Mca concentration

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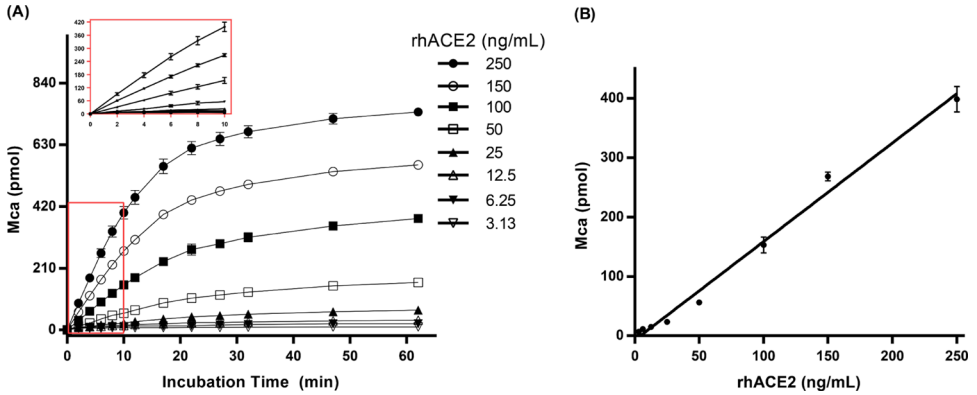

Figure 2. rhACE2 activity toward the substrate Mca-APK(Dnp). (A) Time-dependent increase of the Mca product from the 11.25 μM substrate by rhACE2 at 3.13−250 ng/mL at 37 °C. (B) Dose response of rhACE2 on product formation with the 11.25 μM substrate for 10 min at 37 °C. Linear regression was $R = 0.9901$, $P < 0.0001$. Data were generated from the three independent experiments with triplicates and expressed as means ± SDs.

was used to determine the amount of the final product in pmol of Mca. In this study, 1 RFU = 40.09 pmol of Mca at 37 °C in the presence of 11.25 μM substrate. For routine testing, the rhACE2 enzyme activity of thawed protein aliquots was normalized. Briefly, the rhACE2 stock solution aliquot at 10 μg/mL was diluted in the assay buffer, then incubated with the substrate (final concentration: rhACE2 50 ng/mL, substrate 11.25 μM) for 10 min at 37 °C. Based on the catalytic product as a reference of 1.358 RFU for 50 ng/mL rhACE2 enzyme, the rhACE2 stock solution was then diluted to be equivalent to the reference activity.

Statistical Analysis. All incubations were conducted in triplicate with three independent experiments. Quantitative data are presented as the mean ± standard deviation (SD) or the standard error of the mean (SEM), as specified in the figure legends. Statistical tests were performed using GraphPad Prism 7.0. Two-sided Student's *t*-tests were used for comparisons between two groups, whereas one-way ANOVA or two-way ANOVA with Tukey's post hoc was used for comparisons among multiple independent groups. Significant differences were considered at $P < 0.05$.

RESULTS

Optimization of the rhACE2 Activity Assay. An rhACE2 protein dose response was established from 3.13 to 250 ng/mL with 11.25 μM substrate at 37 °C (Figure 2). After 10 min of incubation with the substrate, enzymatic activity declined (Figure 2A). At 10 min of incubation, the amount of the product formed was linear from 3.13 to 250 ng/mL rhACE2 (Figure 2B). Thus, a 10 min incubation period was used for subsequent experiments.

The intra- and inter-assay reproducibility were determined to test the robustness of the assay (Table 1). The intra-assay coefficient of variance (CV) for RFU with 25, 50, and 100 ng/mL of rhACE2 and the 11.25 μM substrate at 37 °C was 6.04−7.88%, whereas the inter-assay CV was 2.52−9.96%.

Table 1. Intra- and Inter-Assay Variation of rhACE2 Activity[a]

rhACE2 concentration (ng/mL)	intra-assay RFU[b]			inter-assay RFU		
	mean	SD	CV (%)	mean	SD	CV (%)
100	3.88	0.31	7.88	3.97	0.10	2.52
50	1.61	0.10	6.54	1.62	0.04	2.50
25	0.68	0.04	6.04	0.71	0.07	9.96

[a]Activity of rhACE2 was determined at 37 °C for 10 min with the 11.25 μM substrate. [b]Relative fluorescence unit (RFU) values were generated from subtracting the substrate blank from the readings for the corresponding wells. Data are from $n = 3$ experiments with triplicate samples.

Quercetin Inhibits rhACE2 Activity. The reaction rate of 50 ng/mL rhACE2 in the presence of 5−100 μM quercetin was time- and concentration-dependent at 37 °C (Figure 3A). In contrast, the reaction rate of rhACE2 was consistent at 0.100 RFU/min ($P > 0.05$). After 2.5 min, 100 μM quercetin reduced rhACE2 activity to 0.004 ± 0.029 RFU/min ($P < 0.0001$ vs control), while 10 μM quercetin reduced activity to 0.048 ± 0.022 RFU/min ($P = 0.0007$ vs control). Incubation with 5 μM quercetin reduced rhACE2 activity from 2.5 to 8.5 min, but at 10.5 min, the difference became nonsignificant relative to the control ($P > 0.05$). The impact of time on rhACE2 inhibition was greatest for 5 μM quercetin (Figure 3B). To further describe the time dependency of inhibition, the quercetin IC_{50} value was determined at 2.5 and 10.5 min (Figure 3C,D). The rhACE2 IC_{50} of quercetin increased from approximately 4.48 μM after 2.5 min to 29.5 μM at 10.5 min.

Inhibition of rhACE2 was dependent on temperature (Figure 4). rhACE2 activity increased with temperature (Figure 4A). The amount of Mca formed at 10 min was 2.2-fold when temperature increased from 25 to 37 °C. At each temperature, 10 μM quercetin inhibited rhACE2 activity relative to the control (Figure 4B).

Kinetics of Enzyme Inhibition. A kinetic study was performed at increasing substrate concentrations to determine how rhACE2 activity is linked to catalytic efficiency. At the optimal reaction conditions (Figure 2A), activity over a 10 min incubation period was linear over varying protein concentrations, indicating that the reaction rate was stable over this period. The Michaelis−Menten constant K_m and maximum velocity V_{max} were determined by coincubation of 0.7−90 μM of the substrate and 50 ng/mL rhACE2 in the presence of quercetin (10 μM), rutin (10 μM), or DX600 (1 μM), as a positive control (Figure 5, Table 2). Overall, these compounds decreased the affinity of rhACE2 to the substrate (e.g., higher K_m values compared to the rhACE2 control) and lowered the velocity (with the exception of rutin), resulting in a decreased catalytic efficiency (K_{cat}/K_m). DX600 reduced the maximum initial velocity (3.49 ± 0.66 pmol/min) by 74% compared with the ACE2 control (13.3 ± 0.5 pmol/min) and weakened the affinity of rhACE2 for the substrate with a 5.8-fold increased K_m value and a reduced K_{cat}/K_m value. A similar trend was also

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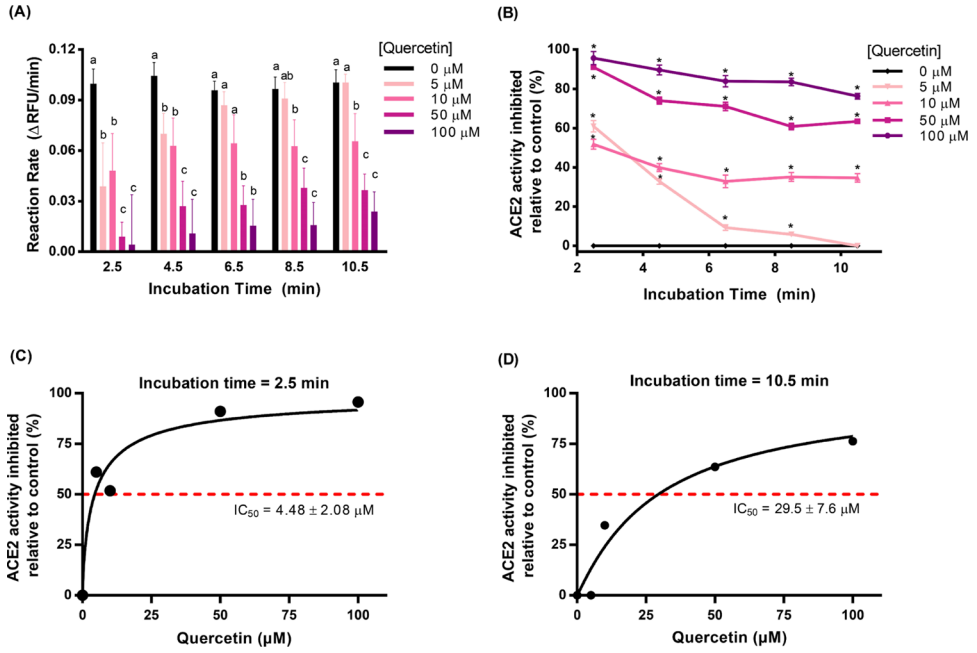

Figure 3. Quercetin inhibits rhACE2 activity. (A) rhACE2 velocity (ΔRFU/min) and (B) rhACE2 inhibition (% of control) in the absence or presence of 5−100 μM quercetin incubation with 50 ng/mL rhACE2, 11.25 μM substrate, for up to 10.5 min at 37 °C. For (A), rate differences were analyzed by two-way ANOVA, followed by Tukey's multiple-comparison test. Incubation time accounted for 5.92% of total variation (P = 0.0002), whereas the quercetin concentration accounts for 78.2% with $P < 0.0001$, however their interaction was not significant ($P > 0.05$). Within each time interval, bars bearing different letters indicate significant differences ($P < 0.05$). For (B), the % inhibition was determined relative to the untreated control at each time point. Differences were assessed by one-way ANOVA, followed by Tukey's multiple-comparison test among different quercetin concentrations at each time interval, *$P < 0.005$ compared to the control. For (A) and (B), the data were obtained by three independent experiments with triplicates and shown as means ± SDs. (C, D) Analysis of rhACE2 inhibition by quercetin concentration at 2.5 min (C) and 10.5 min (D). Data were fitted using a variable slope [inhibitor]-normalized response model in GraphPad Prism 7. Data are represented by three independent experiments with triplicates, and IC_{50} values are means ± SEMs.

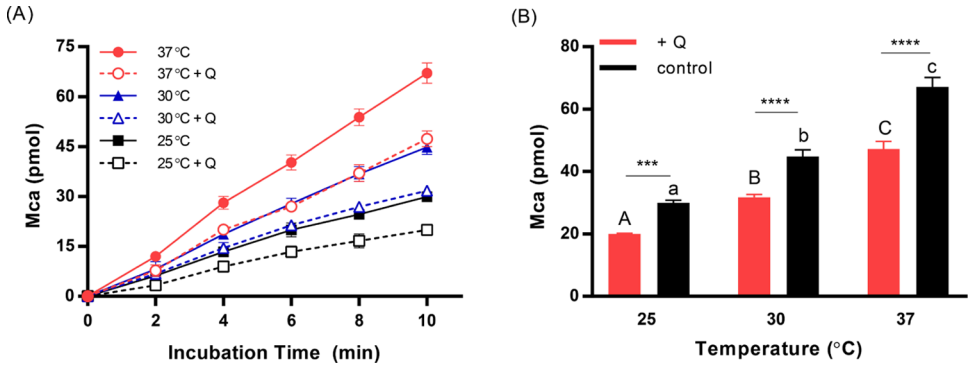

Figure 4. Temperature affects rhACE2 activity. (A) Temperature dependence on the time course of product formation with or without 10 μM quercetin. (B) Effect of temperature on product formation after 10 min of incubation with or without 10 μM quercetin. Assays were performed at 50 ng/mL rhACE2 enzyme concentration with 11.25 μM substrate. Statistical analysis was performed by one-way ANOVA, followed with Tukey's multiple-comparison testing, ***$P < 0.0005$ and ****$P < 0.0001$. Differences between the treated and untreated samples are denoted by upper and lowercase letters, respectively. Data are means ± SDs and are of three experiments performed with triplicate samples.

observed with quercetin, as it had 28% lower V_{max} (9.56 ± 0.36 pmol/min) and 1.7-fold increased K_m compared to the control. For rutin, although the K_m value was lower than rhACE2 alone, the maximum velocity was higher than the rhACE2 control. Thus, the K_{cat}/K_m value for rutin was not similar to quercetin (Table 2).

Polyphenol Inhibition of rhACE2 Activity. Polyphenols with 3′,4′-hydroxylation and known quercetin metabolites (isorhamnetin, tamarixetin, quercetin-3-*O*-glucuronide, and 3,4-dihydroxyphenylacetic acid) were evaluated for rhACE2 inhibition. Nicotianamine, a natural product from soybeans, and an ACE2 inhibitor[19] as well as DX600 were used as controls (Table 3). All of these polyphenols inhibited rhACE2 activity. The extent of rhACE2 inhibition was dependent on time. After 10 min of incubation, the inhibition was significantly decreased relative to 2 min for all polyphenols except (±)-eriodictyol. DX600, nicotianamine, and quercetin were more potent inhibitors than the other tested polyphenols at 2 min. However, at 10 min, quercetin inhibition of rhACE2 was less than that of DX600 and nicotianamine.

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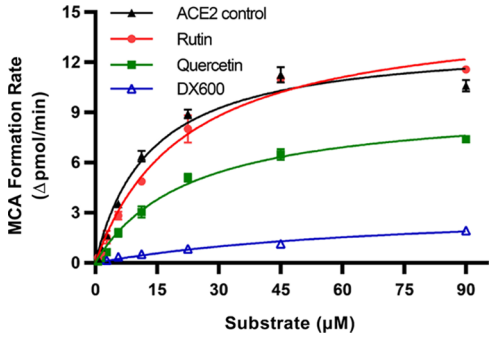

Figure 5. Plot of the rate of Mca product formation (Δpmol/min) as a function of substrate concentration (μM) in a steady-state reaction condition. rhACE2 enzyme (50 ng/mL) was incubated with various concentrations (0.70, 1.41, 2.81, 5.63, 11.25, 22.50, 45, and 90 μM) of the substrate in the presence of 10 μM quercetin, 10 μM rutin, or 1 μM DX600. The absence of quercetin/rutin/DX600 was regarded as the control. The initial velocities of each treatment were obtained by fitting the fluorescence intensity value versus time (0−10 min) data with linear regression. The plot of velocity versus substrate concentration was fitted to the Michaelis−Menten equation to determine the apparent K_m, V_{max}, and K_{cat} constant (listed in Table 2). Fit for control: R^2 = 0.974; rutin: R^2 = 0.986; quercetin: R^2 = 0.987; and DX600: R^2 = 0.949. The fitted curves were different from each other (P < 0.0001). Results are from three independent experiments with triplicates. Data present means ± SDs.

DISCUSSION

Angiotensin-converting enzymes, ACE and ACE2, share considerable homology with 41.8% sequence identity at the catalytic domain, and both belong to the M2 family of metallopeptidases with HEMGH zinc-binding motifs as an amino-terminal catalytic domain.[9,20−22] Despite these similarities, their structures enable different substrate specificity and peptidase activity in the RAAS.[8,9,23,24] Structural and functional studies have revealed that flavonoids inhibit ACE because of the double bond between C2 and C3 on the C-ring; the ketone group of C4 at the C-ring; and the 3′,4′-catechol group in the B-ring.[25−27] Although the ACE inhibition mechanism by flavonoids has not yet been fully understood, the catechol group in the B-ring may exert a charge−charge interaction with the Zn^{2+} ion in the ACE active site.[26] Specifically, luteolin exhibits the highest capacity to inhibit ACE activity among 17 flavonoids with an IC_{50} value of 23 μM, followed by quercetin with an IC_{50} value of 43 μM.[26]

Under optimized assay conditions, quercetin inhibited rhACE2 activity by reducing its affinity for the Mca-APK(Dnp) substrate and led to a lower catalytic efficiency (K_{cat}/K_m). In addition, quercetin had mixed rhACE2 inhibition as it reduced V_{max} and increased K_m. Structurally related polyphenols also inhibited rhACE2. Quercetin metabolites (isorhamnetin, tamarixetin, 3,4-dihydroxyphenylacetic acid, and quercetin-3-glucuronide), rutinosides and glycosides of quercetin, and other flavonoids (luteolin, (±)-eriodictyol, and (−)-epicatechin) also inhibited rhACE2. Notably, polyphenols were less potent inhibitors than DX600. However, flavonoids still decreased K_m and K_{cat}/K_m values, indicating interaction with the ACE2 active site. Further analysis by computational simulation, X-ray crystallography, or NMR needs to be employed to define the specific interactions of flavonoids with 3′,4′-dihydroxylation and ACE2.

Quercetin is mainly distributed in foods as glycosides and rutinosides. It is metabolized by deglycosylation, glucuronidation, sulfation, methylation, or further catabolism by gut microbiota to phenolics such as 3,4-dihydroxyphenylacetic acid. In humans, quercetin intake results in C_{max} values of ≤10 μM.[28] Microbial quercetin metabolites are more abundant, presumably because other flavonoids are also metabolized to these phenolic acids. For example, fasting serum levels of 3,4-dihydroxyphenylacetic acid have been reported as high as

Table 3. Polyphenols Inhibit rhACE2 Enzyme Activity[a]

class	polyphenol	inhibition (%)[b] 2 min	inhibition (%)[b] 10 min
flavonols	quercetin	66.2 ± 2.2^{A}	38.1 ± 1.9^{A,*}
	quercetin-3-*O*-glucoside	47.7 ± 3.7^{B}	20.9 ± 2.4^{B,*}
	quercetin-3-*O*-galactoside	34.2 ± 3.7^{C}	12.9 ± 2.8^{C,*}
	rutin	48.3 ± 4.7^{D}	14.5 ± 2.7^{D,*}
	quercetin-3-*O*-glucuronide	33.1 ± 4.9^{E}	10.2 ± 3.3^{E,*}
	tamarixetin	41.5 ± 5.0^{F}	19.6 ± 3.2^{F,*}
	isorhamnetin	14.7 ± 1.4^{G}	12.2 ± 0.5^{G,*}
flavanones	(±)-eriodictyol	24.4 ± 1.4^{H}	25.6 ± 0.8^{H}
flavanols	(−)-epicatechin	27.4 ± 5.7^{I}	4.39 ± 3.06^{I,*}
flavones	luteolin	37.1 ± 0.6^{J}	26.1 ± 1.7^{J,*}
phenolic acids	3,4-dihydroxyphenylacetic acid	42.1 ± 3.4^{K}	6.48 ± 2.61^{K,*}
known ACE2 inhibitors	nicotianamine	64.5 ± 0.5^{A}	81.2 ± 0.1^{L,*}
	DX600	67.7 ± 1.3^{A}	80.3 ± 1.3^{L,*}

[a]Screening experiments were performed by coincubation of 50 ng/mL rhACE2 enzyme and 11.25 μM substrate at 37 °C for 2 or 10 min in the presence of 10 μM of compounds, except for 1 μM of DX600. [b]Statistical analysis was by one-way ANOVA with the Dunnet post hoc test, with P < 0.05 considered significant, denoted by uppercase letters. Two-tailed t-tests were conducted for each inhibitor to evaluate differences by time, denoted by * at 10 min, where P < 0.05 was considered significant. Data are means ± SDs from three individual experiments with triplicates.

Table 2. Steady-State Kinetic Parameters of 10 μM Rutin, 10 μM Quercetin, and 1 μM DX600 with rhACE2[a]

treatment	V_{max} (pmol × min^{-1})[b]	K_m (μM)[b]	K_{cat} (s^{-1})[c]	K_{cat}/K_m (μM^{-1} s^{-1})
rhACE2	13.3 ± 0.5	13.2 ± 1.6	3.78 ± 0.15	0.29
rhACE2 + rutin	15.1 ± 0.6	21.6 ± 2.1	4.28 ± 0.16	0.20
rhACE2 + quercetin	9.56 ± 0.36	22.9 ± 2.2	2.71 ± 0.10	0.12
rhACE2 + DX600	3.49 ± 0.57	76.5 ± 21.9	0.989 ± 0.156	0.013

[a]50 ng/mL rhACE2 enzyme was incubated with various concentrations (0.70, 1.41, 2.81, 5.63, 11.25, 22.50, 45, and 90 μM) of the substrate in the presence of 10 μM quercetin, 10 μM rutin, or 1 μM DX600. Results represent triplicates from three independent experiments. The V_{max}, K_m, and K_{cat} values were expressed as means ± SEMs. [b]K_m and V_{max} were determined by fitting the velocities to the Michaelis−Menten relationship by nonlinear regression. [c]Turnover number K_{cat} was calculated from the V_{max} value ($K_{cat} = V_{max}/[E]$), using a molecular mass of 85 kDa for rhACE2.

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~100 μM.[29] ACE2 is primarily a membrane-bound enzyme located in the heart, lungs, vasculature, kidneys, and oral and digestive tracts.[30] In rats, quercetin and its methyl metabolites accumulate to nmol/g (dw) concentrations in the lung, kidney, heart, and muscle after consumption of 1% quercetin for 11 weeks.[31] After a single dose of [2-^{14}C]quercetin-4′-glucoside to rats, 71% of the dose was recovered in the GI tract at 6 h, which later declined to 3.4% by 24 h.[32] Thus, ACE2 inhibition is plausible in the oral cavity and digestive tracts.

Increased flavonoid consumption is associated with reduced mortality from cardiovascular diseases in older women.[33] Meta-analyses of randomized controlled trials have associated the intake of >500 mg quercetin per day with reduced blood pressure.[34,35] Yet the precise antihypertensive mechanism of quercetin is still not clear,[36−38] partly due to the variation of administration dosage, experimental duration, type of experiment design, and participant population.[39] *In vitro* studies have demonstrated that flavonoid-rich foods are capable of inhibiting ACE activity.[40−42] Also, an acute hypertensive rat model supports the antihypertensive potential of quercetin through reduced plasma ACE activity.[43] In contrast, ACE inhibition by flavonoids has not been observed in other human and animal studies.[44−47] On the bias of homology between ACE and ACE2, it would be expected that inhibition of ACE2 by quercetin would be independent of its ability to modulate blood pressure. Further mechanistic studies are needed to define the impact of polyphenol ACE2 inhibition on the RAAS.

Inhibition of ACE2 may be undesirable because functional ACE2 inhibits inflammation by reducing activation of the angiotensin II type 1 receptor pathway.[15] SARS-CoV-2 uses ACE2 as a receptor to enter cells, and the resulting proteolysis of ACE2 contributes to lung damage.[15,48] While disrupting S protein and ACE2 interactions might prevent SARS-CoV-2 entry to cells, inhibiting ACE2 activity could be detrimental to infection recovery. Therefore, it is important to clarify if polyphenol−ACE2 interactions inhibit S-protein binding and ACE2 activity in tissues. If both occur, it will be important to understand if the antioxidant and anti-inflammatory activity of polyphenols through other pathways (e.g., inhibition of nuclear factor κB and activation of nuclear factor erythroid 2-related factor 2) would negate the proinflammatory aspects of ACE2 inhibition during SARS-CoV-2 infection.

In summary, polyphenols with 3′,4′-dihydroxylation inhibit rhACE2 activity *in vitro*. Among the polyphenols tested, quercetin was the most effective rhACE2 inhibitor, and several of its known metabolites also function as inhibitors. In the context of flavonoid metabolism, it is plausible that dietary polyphenol intake could inhibit ACE2, particularly in the digestive tract. Given these findings, it is urgent to further investigate the functional effects of polyphenols on ACE2 *in vivo*.

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ABBREVIATIONS

SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; S protein, spike glycoprotein; RAAS, renin−angiotensin−aldosterone system; rhACE2, recombinant human angiotensin-converting enzyme 2; ACE, angiotensin-converting enzyme; Ang II, angiotensin II; RFU, relative fluorescence unit; Mca, 7-methoxycoumarin-4-acetic acid

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